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Glucose-Mediated Catabolite Repression of the Tricarboxylic Acid Cycle as an Explanation for Increased Acetic Acid Production in Suicidal *Aeromonas* Strains

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Growth in the presence of glucose, even under highly aerobic conditions, significantly reduced the activities of three tricarboxylic acid cycle enzymes, citrate synthetase, α -ketoglutarate dehydrogenase, and malate dehydrogenase, in suicidal but not nonsuicidal *Aeromonas* strains. Pyruvate dehydrogenase activity, however, was significantly increased. The activities of all of the enzymes, as well as the glucose-mediated increase in acetic acid production, were shown to be regulated by catabolite repression. The regulator protein is the same one which regulates the utilization of several sugars.

In an earlier paper (11), we described a phenomenon in which certain strains of *Aeromonas* spp., including all *Aeromonas caviae* and some *A. sobria* isolates, were not recoverable after 24 h when grown in nutrient broth supplemented with glucose. It was shown that death of the cells was due to the accumulation of acetic acid, which is produced in large quantities by these strains even when the cultures are incubated under highly aerobic conditions. The phenomenon was termed "suicide," and the requirements for its manifestation were consistent with the absence of these *Aeromonas* biotypes in acidic lakes in New England and their recovery from alkaline waters in Israel and from sewage at both locations. In this paper, we examine the possibility that the increased production of acetic acid by suicidal aeromonads was achieved by a shutdown of the tricarboxylic acid (TCA) cycle and the diversion of acetyl-coenzyme A produced from pyruvate to the production of acetic acid. We also identify the regulatory mechanism.

Production of acetic acid. Accumulation of acetic acid in nutrient broth-glucose (NBG) (11) shake cultures of the nonsuicidal strains was greatest during the lag period, increased only slightly when the growth and glucose utilization rates were maximal, and then decreased, presumably because acetate was used as an energy source once the glucose was metabolized (Fig. 1A). In the suicidal cultures (Fig. 1B), however, acetic acid continued to be produced until both growth and glucose utilization were prematurely inhibited by the accumulation of acetic acid in its un-ionized form (11). About 43% of the glucose catabolized by the suicidal strains and only 8 to 12% of that used by the nonsuicidal isolates could be accounted for by the acetic acid in the cultures. When the pH of the suicidal cultures was maintained between 6.5 and 7.0, however, the optical density reached a maximum of 1.8, all of the glucose was metabolized, and considerably more acetate was produced, with most of it appearing early in the exponential growth phase (Fig. 2A). *Escherichia coli* resembled the suicidal aeromonads in that high acetate levels also were produced, there was a sharp

decrease in pH after 3 h, and about 45% of the glucose metabolized could be accounted for by the acetic acid produced. With *E. coli*, however, most of the acetic acid was produced as the cell population was passing into the stationary phase, and there was little, if any, acetic acid-mediated death of the cells.

Inhibition of TCA cycle enzymes and stimulation of pyruvate dehydrogenase activity. The specific activities of three enzymes in the TCA cycle, malate dehydrogenase (18), α -ketoglutarate dehydrogenase (8), and citrate synthetase (13), in cell-free extracts from Luria broth (LB) cultures were essentially the same whether the nonsuicidal strains were grown in the presence or absence of added glucose or when the suicidal strains were grown in its absence. However, when the suicidal strains were grown in LB supplemented with glucose, the specific activities of all three enzymes were appreciably and significantly decreased (Table 1). LB with glucose, which has a greater buffering capacity and less glucose (0.3%), was used instead of NBG to minimize acetic acid-mediated death of the cells. Pyruvate dehydrogenase activity also was similar with the nonsuicidal strains grown in the presence or absence of glucose and the suicidal strains grown in its absence. When glucose was added to the cultures of the suicidal strains, however, pyruvate dehydrogenase activity was significantly increased rather than decreased (Table 1).

Effect of cAMP on activities of the enzymes and acetic acid production. The addition to LB with glucose of filter-sterilized cyclic AMP (cAMP) at a final concentration of 3×10^{-2} M reversed the glucose-mediated inhibition of the activities of the three TCA cycle enzymes and its stimulation of pyruvate dehydrogenase activity in suicidal aeromonads (Table 1). The activities of the four enzymes in the extracts from the cultures of the nonsuicidal strains were not significantly altered by the addition of cAMP. Moreover, the addition of cAMP to NBG shake cultures of suicidal *Aeromonas* strains reduced acetic acid production, increased the pH, and, because of this, prevented acetic acid-mediated death of the cells (Fig. 2B).

Identity of the catabolite repressor protein. Mutants which simultaneously lost the abilities to utilize lactose, arabinose, and galactose in cAMP-containing media were sought by

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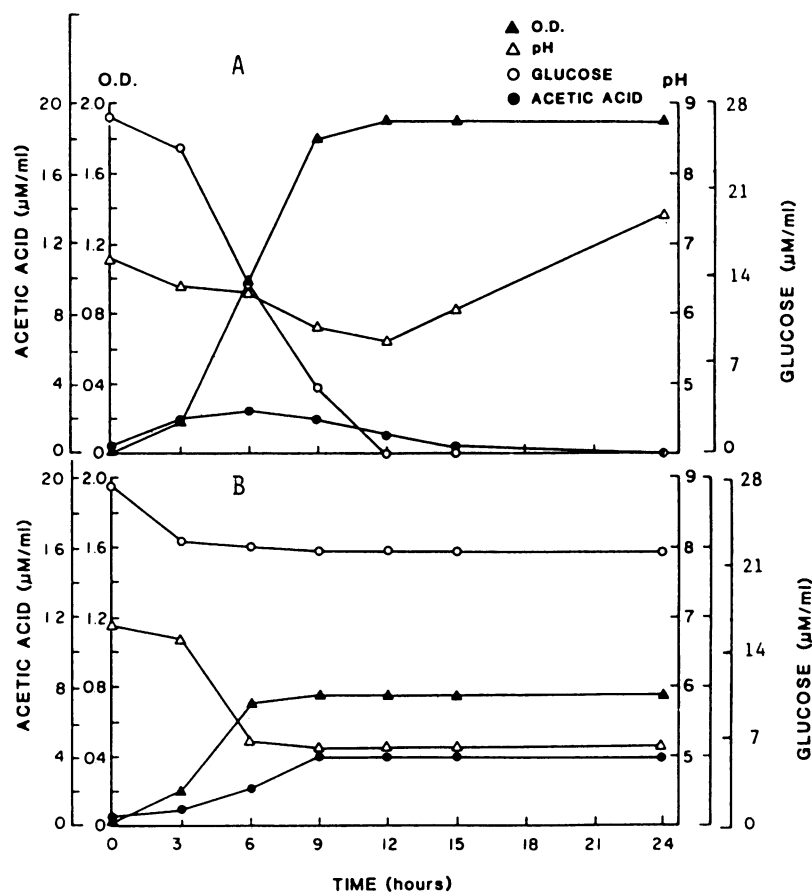


FIG. 1. Acetic acid production relative to glucose utilization, growth as optical density (O.D.), and pH in NBG shake cultures incubated aerobically. (A) Nonsuicidal *Aeromonas* strain (2BT); (B) suicidal *Aeromonas* strain (OP2).

nitrosoguanidine mutagenesis (3). Two types of mutants were obtained (6, 14) (Table 2). Adenylate cyclase (Cya^-) mutants fermented the three sugars only upon the addition of cAMP to the media, while " Crp^- " mutants did not ferment the sugars even when cAMP was added. The presumed Crp^- mutants, in contrast to the wild-type strain, remained suicidal even when cAMP was added to the media. The uptake of cAMP by the presumed Crp^- mutants was examined, since a loss of the ability to transport cAMP would have produced similar findings. The uptake of [3H]cAMP by the CRP mutants was not significantly different from that of the Cya^- mutants or the parental strain, OP2.

Catabolite repression of the TCA cycle enzymes leading to the production of acetic acid under highly aerobic conditions and depression by cAMP also occurs in *E. coli* (1, 7, 9, 10, 18). *E. coli* differs from the suicidal aeromonads, however, in that oxidative phosphorylation and the synthesis of cytochromes also are repressed (2, 5), and the acetic acid is produced late rather than early in the growth cycle.

The increase in pyruvate dehydrogenase activity in *A. caviae* would not only increase the production of acetic acid but also compensate somewhat for the loss in energy due to the glucose-mediated catabolite repression of the TCA cycle. Since neither succinate nor propionate is produced in the shake cultures (11), we conclude that oxidative phosphorylation remains operative and that the increase in pyruvate dehydrogenase activity increases the energy available from the oxidation of the pyruvate to acetyl-coenzyme A. Since the production of pyruvate is regulated by the levels of

AMP and ADP in the cells (17) and pyruvate in turn regulates the synthesis of the pyruvate dehydrogenase complex (4), we speculate that the glucose-mediated increase in pyruvate dehydrogenase activity is secondary to the partial shutdown of the TCA cycle.

TABLE 1. Effects of glucose on enzyme activities and reversal by cAMP in suicidal strains of *Aeromonas* spp.

| Enzyme ^a | <i>Aeromonas</i> type | Sp act in cells grown aerobically with ^b : | | |
|---------------------|-----------------------|---|---------|----------------|
| | | No glucose | Glucose | Glucose + cAMP |
| MDH | Suicidal | 15.7 | 3.3* | 14.7 |
| | Nonsuicidal | 15.1 | 12.5 | 15.1 |
| KDH | Suicidal | 10.7 | 2.4* | 11.0 |
| | Nonsuicidal | 11.5 | 11.2 | 11.5 |
| CSN | Suicidal | 1,164 | 209* | 1,170 |
| | Nonsuicidal | 948 | 955 | 1,123 |
| PDH | Suicidal | 216 | 950* | 167 |
| | Nonsuicidal | 205 | 264 | 215 |

^a MDH, Malate dehydrogenase; KDH, α -ketoglutarate dehydrogenase; CNS, citrate synthetase; PDH, pyruvate dehydrogenase.

^b Means from four assays (duplicate assays on extracts from two strains). Specific activity is expressed as micromoles of substrate converted per minute per milligram of protein for MDH, KDH, and PDH and as nanomoles of substrate converted per minute per milligram of protein for CSN. *, Significantly different from nonsuicidal strain, when glucose was omitted or cAMP was added, at $P < 0.01$.

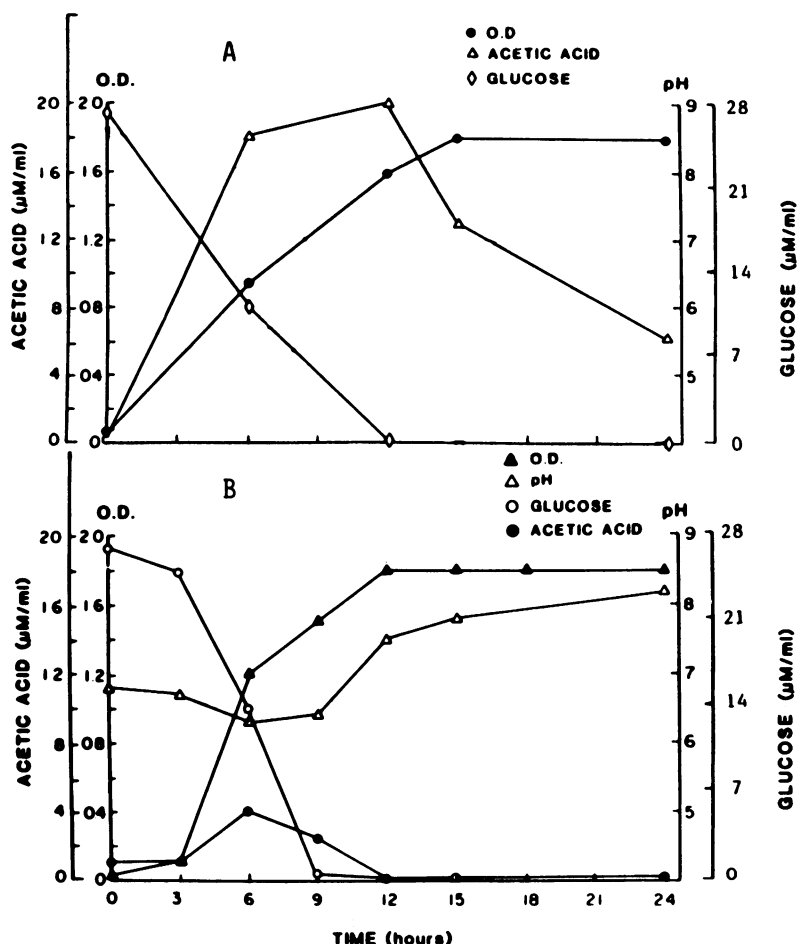


FIG. 2. Acetic acid production relative to glucose utilization, growth as optical density (O.D.), and pH in 30°C NBG shake cultures of the suicidal *Aeromonas* sp. strain OP2. (A) pH maintained between 6.5 and 7.0; (B) cAMP added to medium at a final concentration of 3×10^{-2} M.

The metabolism of *A. caviae* appears to be extremely well adapted to growth in calcium-rich alkaline environments such as those from which most of the strains were isolated. Unlike the nonsuicidal aeromonads, *A. caviae* can grow anaerobically in a glucose-mineral salts medium (11), is anaerogenic, and does not produce 2,3-butanediol as a fermentation end product (15). Also, it utilizes a number of β -glycosides, including cellobiose, esculin, salicin, and lactose; this is due in part to the presence of a β -glycosidase with broad specificity for β -glucosides and β -galactosides (M. Rodgers, personal communication). The most probable explanation for the adaptation towards the

production of acetic acid by *A. caviae* is the mobilization of soluble phosphate from insoluble $\text{Ca}_3(\text{PO}_4)_2$. A second possibility is that the acetic acid acts to liberate nutrients from associated algae. These possibilities need to be examined ecologically.

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TABLE 2. Fermentation and suicide reactions of Crp^- and Cya^- mutants in the presence of cAMP

| Mutant type | No. of isolates examined | Fermentation of Lac, Ara, Gal ^a | Suicide ^a |
|----------------|--------------------------|--|----------------------|
| Crp^- | 3 | — ^b | + |
| Cya^- | 5 | + ^b | — |
| Parent | | + | — |

^a Mutants and parent ferment glucose with and without cAMP and are SUIC⁺ without cAMP.

^b Lactose (Lac), arabinose (Ara), and galactose (Gal) were not fermented in the absence of cAMP.

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